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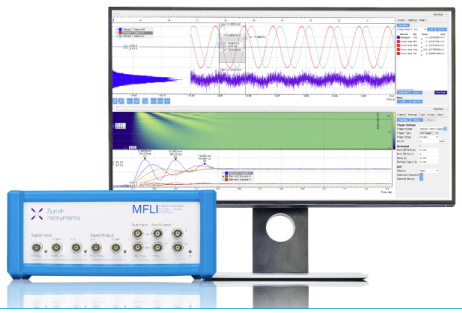

PREFACE: Proceedings of the 2nd International Conference on Biosciences and Medical Engineering (ICBME2019)

The 2nd International Conference on Biosciences and Medical Engineering 2019 (ICBME 2019) organised by Universiti Teknologi Malaysia (UTM) and Udayana University (UNUD) was successfully held in one of the most popular Island of Bali between 11-12 April 2019. The scientific event had brought together scientists from many countries of various research areas to present their latest scientific findings that may prove useful for spurring future potential collaborative endeavour. The theme that emphasised on collaborative and multidisciplinary researches in a plethora of scientific fields that included those of Biosciences, Chemistry, Physics, Mathematics as well as Medical Engineering has extended research boundaries, while embracing the differences and strengths. The two days conference was attended by 160 participants from more than 13 countries namely Algeria, Turkey, Qatar, USA, Pakistan, Philippines, Japan, Nigeria, Sudan, Australia, Iraq, Malaysia and Indonesia during the opening ceremony and keynote lectures. The participants that were mainly faculty members and research students actively engaged in the discussions. The participants presented their most recent technological and scientific findings, suited to the conference theme for this year, "Towards Innovative research and cross-disciplinary collaborations". Keynote speakers of the 2nd ICBME 2019 comprised of well-known and established researchers *viz*. Prof. Emeritus Dr. Chiharu Nakamura of Kobe University, and now serving Ryukoku University in Japan as well as Prof. Dr. Uda Hashim, an expert on Nanotechnology for Medical Diagnosis from Universiti Malaysia Perlis (UniMAP). Plenary speakers were Prof. Dr. Nuri Andarwulan from Bogor Agricultural University, and the other two, were Prof. Dr. Teruo Sone from Hokkaido University, Japan and Prof. Dr. Chung-Ho Lin from the University of Missouri, USA.

I would like to extend my deepest gratitude to the state governments of Pulau Pinang, Selangor, Melaka & Kedah of Malaysia, Yayasan FELDA and ADABI for their financial contributions for the ICBME2019.

This issue of AIP Conference Proceedings is a collection of selected contributions made by participants covering mainly the fields of Biosciences and Medical Engineering. ***The Editorial Board Members*** are deeply indebted to all the authors for their valuable contributions, as well as to the reviewers for their professional inputs and their carefulness and patience.

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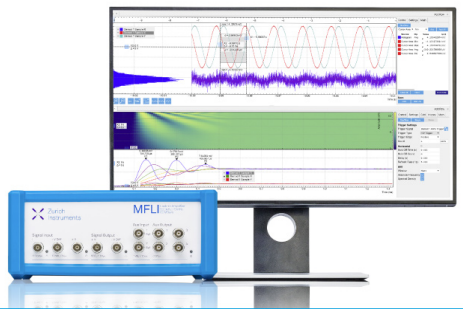

Isolation and *In Vitro* Screening of Plant Growth Promoting Rhizobacteria from Barak Cenana Red Rice

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Abstract. This research aimed to get bacterial isolate from rhizospheric soil of Barak Cenana, one of red rice cultivar. Rhizosphere area is a potential source as plant growth promoting rhizobacteria (PGPR) can be isolated and utilized to support growth and productivity of Barak Cenana red rice in the future. The potential of isolates as PGPR inoculants was determined based on the ability of the isolates to produce siderophores, Indole Acetic Acid (IAA), and phosphate solubilization, by *in vitro* screening. The results suggested that G4c isolate has potential as plant growth promoting ability, based on the promising observation of siderophores production, the highest concentration of Indole Acetic Acid (IAA) production, and phosphate solubilization. Based on 16S rRNA sequencing by using 785F and 907R primer set, G4c isolates were tentatively identified as *Rhizobium pusense*.

INTRODUCTION

Rice is the main carbohydrate source for the majority of Indonesia's citizens. Fertilization is one effort to meet the needs of plant nutrients requirement during its cultivation. The use of inorganic fertilizers in Indonesia is still high because it has affordable and easily obtained prices, but in the long run, it can affect soil quality and environmental condition [1]. The use of bacterial-growth-booster plants can be a solution to improve the quality of plant growth and can help improve soil quality because it has a symbiotic relationship with plants, such as increasing plant growth by producing nutrients and hormones, and also playing a role in activating plant defense systems against pathogens called Induced Systemic Resistance (ISR). The rhizosphere is a layer of soil that covers the surface of plant roots and is influenced by symbiotic activity between bacteria and plant roots. The Rhizosphere is a very good area for the growth of microorganisms because there are exudates produced by plant roots and affect the population of microorganisms, generally more numerous and diverse than those in non-rhizosphere [2]. Microorganisms that played a role in supporting plant growth are known as bacterial-spur-growth-plants or Plant Growth Promoting Rhizobacteria (PGPR). Previous research conducted by Sukweenadhi et al. [3], *Sphingomonas panaciterrae* and *Paenibacillus yonginensis* which obtained from ginseng field soil in South Korea have the potential ability as a PGPR with the ability of isolates to produce IAA, siderophores, and solubilize phosphate. In the research conducted by Ullah et al. [4], PGPR isolates obtained from *Zea mays* were able to produce IAA, gibberellins and also accumulate heavy metals in the soil. In this study, isolation from the rhizosphere area of Barak Cenana red rice and *in vitro* screening of the ability of isolates in producing IAA, siderophores and solubilizing phosphate was done. Barak Cenana red rice is a local rice variety which is planted from generation to generation in the highlands in Penebel District, Tabanan, Bali. Barak Cenana red rice typically required six months to reach the

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harvesting stage which resulting its cultivation happened not more than one cultivation in one [5]. Isolates that have the ability as PGPR can be used as an inoculant in order to make organic fertilizers to produce better effects on plant growth and contribute to improving the quality of agricultural land.

MATERIALS AND METHODS

Soil Preparation

The soil samples used in this study were obtained from the Barak Cenana red rice farm in Jatiluwih village, Penebel, Tabanan, Bali, during September 2018. Soil samples were taken to the Microbiology Laboratory, University of Surabaya, for isolation, purification, and *in vitro* screening of bacteria in the rice rhizosphere area. Soil sample an approximately 1 gram of rhizosphere were mixed into 9 mL of physiological solution (0.9% NaCl), then diluted serially to a dilution rate of 10-6. One ml of suspension from 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were inoculated into a test tube containing semi-solid Nitrogen Free Bromothymol Blue (NFb) media with pH 6.8 then incubated for 2-7 days at 31°C. NFb media was a selection medium for nitrogen-fixing microorganisms. This media does not contain a nitrogen source so that microorganisms that do not have the ability to nitrogen-fixing cannot survive in this medium [6].

Bacteria Isolates Purification

Isolation and purification of bacteria from the rhizosphere area of Barak Cenana red rice were done by inoculating 1 mL of diluted soil samples into semi-solid NFb media, then incubated at 31ºC until pellicles are formed. Colonies that are able to form pellicles on semi-solid NFb media are transferred to solid NFb media with a quadrant streak method. All types of colonies that are able to change the color of NFb media into blue color will be subculture on new solid NFb media to obtain a single colony. Then, the purified colonies will be also stored at 30% glycerol stocks [6].

Gram Staining

Gram staining was done to determine the color and morphology of the isolates. Glass of fat-free object was dropped by NaCl, then isolates from solid media are taken using an ose and placed on NaCl droplets, suspended and left to dry in air. After drying, fixation was done to attach bacteria to the object glass by passing the object glass on the fire, the glass side of the object with the spread of bacteria facing up. Object glass was dripped with gentian violet carbolic solution (Gram I), left for 3 minutes then rinsed with distilled water, then dropped Lugol solution (Gram II), left for 1 minute then rinsed with distilled water. Object glass was rinsed using 96% alcohol (Gram III) slowly until no dye was dissolved, then rinsed with distilled water. At last, object glass was dropped with fuchsin solution (Gram IV), left for 3 minutes then rinsed with distilled water and dried in air. The color and morphology of the bacteria cells were observed using a microscope with 1000x magnification [7].

In Vitro Screening of Plant Growth Promoting Abilities

Testing the production of Indole Acetic Acid (IAA) was carried out to determine the ability of isolates producing IAA. 1 ose of the full pure isolate was grown on Tryptic Soy Broth (TSB) media, incubated for 5 days at 31ºC and sampling every 24 hours. One mL of the isolate was centrifuged at a speed of 5000 rpm for 10 minutes. The measurement of IAA concentration was carried out by reacting 0.5 mL of supernatant isolate and 1 mL of Salkowski's reagent in a dark room for 1 hour, observed using a spectrophotometer at a wavelength of 530 nm. Standard curve of the test was made with testing the absorbance of various concentrations of 1-10 ppm and 10-90 ppm of IAA. Salkowski's reagent was made by dissolving slowly 1.2 g of $FeCl_3$ in 50 mL 7.9 M H_2SO_4. The solution was made when it will be used and stored in a chocolate bottle to avoid damage [3]. The siderophores production test was carried out by concentrating 200 µL of bacterial culture (3 days incubation on TSB at 31ºC) to 50 µL, then 10 µL of the concentrated culture was dropped into King B media, incubated at 31ºC for 1-2 days. Positive results are indicated by the change in the color of the media around the colony to yellow or orange. As much as 9.25 g of King's B agar and 3.75 mL of 1.5% glycerol, mixed in 225 mL of distilled water. Addition of

15.125 mg of chrome azurol S in 12.5 mL of distilled water, 18.23 mg of hexadeciltrimethyl (CTAB) in 10 mL of distilled water and 2.5 mL of iron (III) solution in sequence, then heating until the media was clear and boiling, sterilization was carried out use autoclave at 121ºC for 20 minutes [3]. Phosphate solubilization test was carried out by concentrating 200 µL of bacterial culture to 50 µL, then 10 µL of the concentrated culture was dropped into 1/3 Pikovskaya solid media, incubated at 31ºC for 3 days. Positive results are indicated by the formation of clear zones around the isolates [3].

PCR of 16S rRNA

One stab of the isolate using a sterile toothpick was dissolved in 50 µL ddH_2O sterile in a microtube. Cells were lysed by heating at 95°C for 5 minutes, then cooled at room temperature for 5 minutes [8]. The PCR composition used in this study was presented in Table 1 with a total volume of PCR reactions of 55 µL. A total volume of 5 µL of PCR results was used for electrophoresis and 50 µL of PCR results were used for the sequencing stage. Microtube contains a mixture of PCR components included in the PCR by setting the temperature and time of denaturation, annealing, and extension sequentially was 94°C for 45 seconds, 60°C for 30 seconds, and 72°C for 90 seconds, for a total of 40 cycles [9].

TABLE 1. PCR Mix of the 16S rRNA Gene Amplification

PCR Components		Volume
Master Mix		27,5 µL
Primer	*Forward*	5 µL
	Reverse	5 µL
Template (targeted DNA)		15 µL
ddH_2O		2,5 µL
The total volume of reaction		55 µL

16S rRNA Sequencing

16S rRNA sequencing was carried out by using forward 27F 5 'primer (TGA TCM TGG CTC AG TGA GTT) 3' and reverse 1492R 5 '(ACG ACT TAC GGY TAC CTT GTT T) 3' primers [10]. The PCR reaction was added with 20 ng of the genomic DNA as a template in the reaction mixture of 30 µL using EF-Taq (SolGent, Korea) as follows: Taq polymerase activation at 95°C for 2 minutes, 35 cycles of 95°C for 1 minute, 55°C and 72°C for 1 minute each, ending with a 10 minutes of 72°C. Amplification products were purified with multiscreen filter plates (Millipore Corp, Bedford, MA, USA). Sequencing reactions are carried out using the PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. DNA samples containing extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, Genomics Macrogen, South Korea). The mixture was incubated at 95°C for 5 minutes, followed by 5 minutes on ice and then analyzed by the ABI Prism 3730XL DNA Analyzer (Applied Biosystems, Foster City, Genomics Macrogen, South Korea).

Sequencing Results Identification

The sequenced results were then compared to the gene sequence listed in the NCBI data base using a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The target DNA sequence was entered in the white column that was provided, option 16s ribosomal RNA sequences (Bacteria and Archaea) were selected, then click BLAST. The top BLAST results were sequences with the highest similarity. Another reliable database selected in this identification was EzBioCloud website (https://www.ezbiocloud.net/). From both databases, the similarity of 16S rRNA sequences was identified from the best score of the compared species or strains.

RESULTS AND DISCUSSION

The results of colony morphology and Gram staining results of the isolated colonies from the rhizosphere of Barak Cenana red rice, as well as *in vitro* test of its plant growth promoting bacteria, such as IAA production, siderophores production, and phosphate solubilization were presented in the study. In the end, the 16S rRNA sequencing results were also provided. NFb media was used as the initial selection medium, the presence of nitrogen-fixing bacteria was detected by the formation of white pellicles and the changes to blue media (Figure 1), in which due to production of alkaline ammonium. Color changes in the media were caused by the pH bromothymol blue (BTB) indicator on the media. The alkaline condition makes the blue color appears as the positive results of nitrogen fixation.

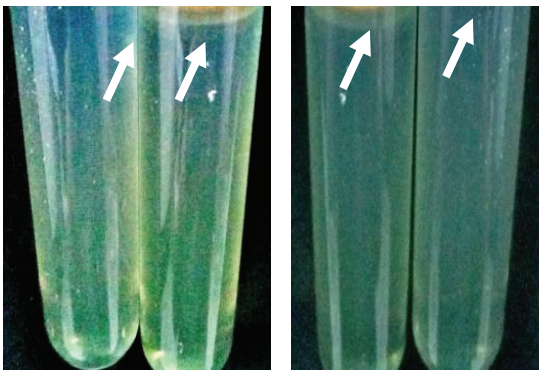


FIGURE 1. Semi-Solid NFb Media Color Change. An arrow indicates a formed blue pellicle.

From the results of isolation and purification, 10 pure single isolates were obtained by coding G1, G2, G3, G3a, G3b, G3c, G4c, G4a_p, G4a_a, and G4b_p isolates. Colony morphology and Gram staining results are presented in Table 2 below. The majority of isolates obtained were Gram-negative, rod-shaped bacteria.

TABLE 2. The Colony Morphology and Gram Staining Results of Bacteria Isolates from the Rhizospheric Soil of Barak Cenana Red Rice

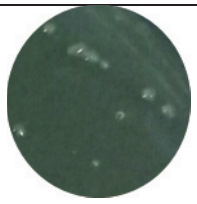
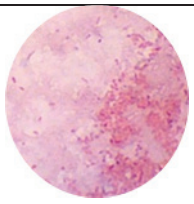
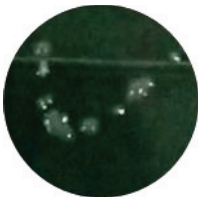
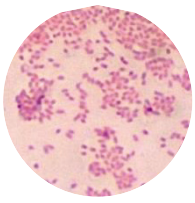
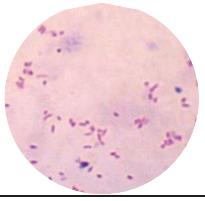
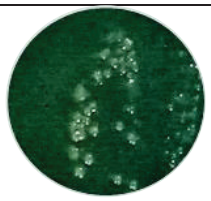
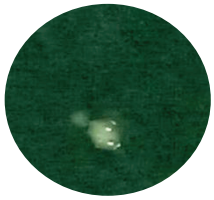
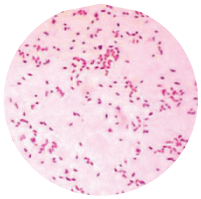
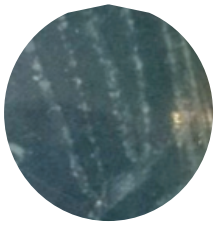
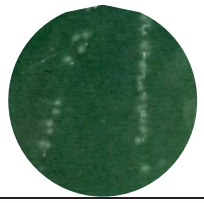
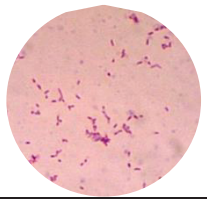
Isolates	Colony Morphology		Gram Staining (1000x magnification)	
G1		Very small, transparent, convex, round, flat edges		Negative, rod, short
G2		Very small, white, convex, flat edges		Negative, small, coccobacillus
G3		Small, white, convex, round, flat edges		Negative, rod, small

TABLE 2. The Colony Morphology and Gram Staining Results of Bacteria Isolates from the Rhizospheric Soil of Barak Cenana Red Rice

Isolates	Colony Morphology		Gram Staining (1000x magnification)	
G3a		White to rather transparent, round, convex, flat edges		Negative, rod
G3b		White, round, convex, flat edges		Negative, comma, small
G3c		White, clearer edges, smaller, convex, flat edges		Negative, coccobacillus, small
G4c		White, round, convex, flat edges		Negative, coccobacillus, small
$G4a_p$		White, convex, flat edges		Negative, rod, short
$G4a_a$		Gray, convex, flat edges		Negative, rod, short, small
$G4b_p$		White, convex, flat edges		Negative, rod, tend to cluster

In vitro screening usually carried out to determine the ability of isolates as PGPR to produce phytohormones, to increase the nutrient intake, and improve plant defense systems in a controlled environment. In this research study, *In vitro* screening was done included indole-3-acetic acid (IAA) production as a parameter representation to see the ability of isolates to produce phytohormones, siderophores production tests as representative parameters to see the ability of isolates to improve plant defense systems, and phosphate solubilization tests as parameter representation to see the ability of isolates to increase nutrient intake of plants. In this study, the results of IAA production testing became the main determining parameter to determine the isolates with potential as plant-growth-boosting agents, followed by results of the ability of siderophores production isolates, and phosphate dissolution tests.

The production test for Indole Acetic Acid (IAA) was conducted to determine the ability of bacteria to produce auxin-type phytohormones. The IAA production test results are presented in Figure 2. On the first to the third day, G4c isolates had the best ability to produce auxin, while G3b and G4a$_p$ isolates had the lowest ability compared to other isolates. After supported by ANOVA, it is known that G4c isolates have the best auxin production ability with a significant difference in auxin production compared to other isolates. But on Day 2, G4c isolates had the best auxin production ability but were not significantly different from G3a isolates. On Day 4 to 5, the best IAA production ability was shown by G3 isolates, but IAA production by G3 isolates was not significantly different from G4c isolates on Day 5. Based on the results of testing conducted for 120 hours, it is suspected that G3 and G4c isolates have the potential to be plant-growth-boosting agents because G3 isolates have the best ability in producing IAA on Day 4 and 5, while isolates G4c have more stable capabilities in producing high concentrations of IAA.

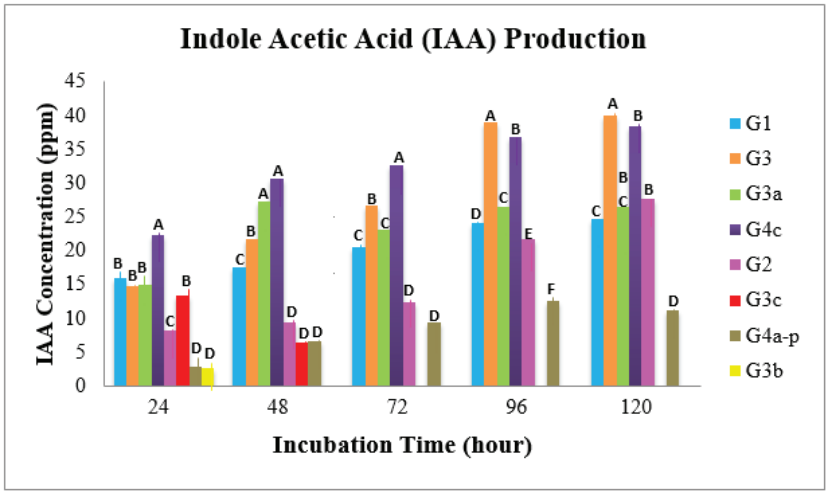


FIGURE 2. Graph of the test results on the production of Indole Acetic Acid (IAA).

The best production of IAA was shown by G3 and G4c isolates.

The results of the siderophores production test are presented in Figure 3. Based on the qualitative test results, it is known that all pure isolates have the ability to produce siderophores, marked by changes in the color of the media around the area where the bacterial culture is pressed into orange (shown arrow).

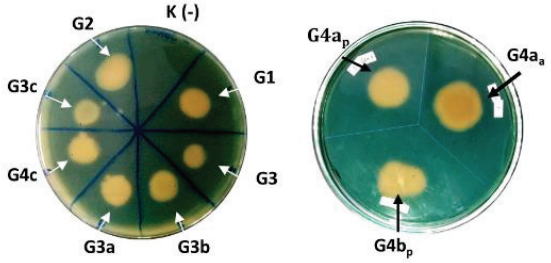


FIGURE 3. The results of the siderophores production test with incubation time for 3 days on modified King B Media showed positive results, the area with bacterial culture was changed to orange. K (-) is a negative control.

The phosphate solubilization test was carried out to determine the bacterial culture's ability to dissolve inorganic phosphate. The results of the phosphate dissolution test are presented in Figure 4. The ability of isolates in dissolving phosphate was detected by the formation of clear zones around the colonies. G1 and G3 isolates do not have the ability to dissolve phosphate, whereas other isolates have the ability to dissolve phosphate which is characterized by the formation of a clear zone around the colony (shown by an arrow).

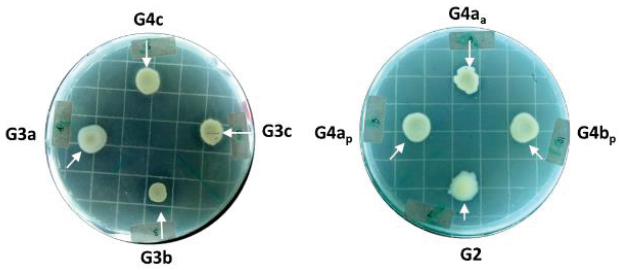


FIGURE 4. The results of phosphate solubilization test with incubation time for 3 days on 1/3 Pikovskaya media showed positive results, formed a clear area around the colony. G1 and G3 isolates do not have the ability to dissolve phosphate, whereas other isolates have the ability to dissolve phosphate which is characterized by the formation of a clear zone around the colony (shown by an arrow).

The testing of the ability of pure single isolates as plant growth promoting ability was carried out by *in vitro* screening which represented the characters of plant growth-promoting rhizobacteria, including the production test of Indole Acetic Acid (IAA), siderophores production test, and phosphate solubilization test. In this study the ability of isolates to produce IAA varied (Fig. 2), possibly influenced by culture conditions, growth phases, and substrate availability [11]. Based on the phosphate solubilization test parameters, G3 and G1 isolates did not show clear zones in the media around the colony (Fig. 4), these data showed that the isolates were unable to dissolve phosphate from tricalcium phosphate ($Ca_3(PO_4)_2$), which is a soluble phosphate, on Pikovskaya media. Thus, it is estimated that isolates G1 and G3 have less potential as a growth agent for plant growth. In testing the ability of isolates to produce siderophores, all isolates showed positive results (Fig. 3) with the occurrence of media color changes to orange due to the elimination of Fe from media dyes [12]. The summary of all isolates plant growth promoting traits is shown in Table 3 below.

TABLE 3. The Summary Results of *In vitro* Screening of Plant Growth Promoting Bacteria Isolates

Isolates	IAA Average Concentration (PPM)					Phosphate Solubilization	Siderophore Production
	24 hour	48 hour	72 hour	96 hour	120 hour		
G1	9,66	9,77	12,38	15,79	16,31	-	√
G3	8,9	13,48	18.16	29,61	31,23	-	√
G3b	1,7		-*			√	√
G3a	9,1	18,78	14,86	17,95	18,09	√	√
G4c	14,1	21,91	23,76	27,51	29,06	√	√
G3c	8,12	3,96		-*		√	√
G2	4,99	5,75	7,45	13,62	19,16	√	√
G4a$_p$	1,79	4,01	5,75	7,67	6,8	√	√
G4a$_a$			-*			√	√
G4b$_p$			-*			√	√

G1 isolate has the ability to produce IAA consistently during 5 days of incubation, and G3 isolates are able to produce high concentrations of IAA. G1 and G3 isolates also have the ability to produce siderophores but do not have the ability to solubilize phosphate. Thus, it is estimated that G1 and G3 isolates have less potential as PGPR ability. G4a$_a$ and G4b$_p$ isolates are estimated to be less potential as a plant growth booster agent because they do not have the ability to produce IAA. G3b and G3c isolates have the ability to produce siderophores and solubilize phosphates. However, G3b and G3c isolates are estimated to be less potential as plant growth promoting agents because G3b isolates are able to produce IAA only on the first day, while G3c isolates are only on the first and second days. The possibility of indole produced by isolates mineralized or transformed into other compounds (such

as indole-3-glyoxylic acid and indole-3-aldehyde acid) with carbon sources, IAA degradation by G3b isolates into other compounds such as indol-3-methyl acid, catechol, genetic acid, and anthranilic acid [13]. Isolates G3a, G2, and G4a_p have the ability to produce siderophores, IAA, and phosphate solubilization. But the concentration of IAA produced by G3a, G2, and G4a_p isolates was not as high as that produced by either G3 or G4c isolates. Isolates G3a, G2, and G4a_p have the potential as a plant growth booster agent, but below the ability of G4c isolates which are estimated to have great potential as plant growth promoting agents based on the results of testing the ability of isolates that always consistently produce high concentrations of IAA, producing siderophores and phosphate dissolution. The ability of G4c isolates to produce high concentrations of IAA consistently is expected to help plant growth, especially in stimulating plant root lengthening.

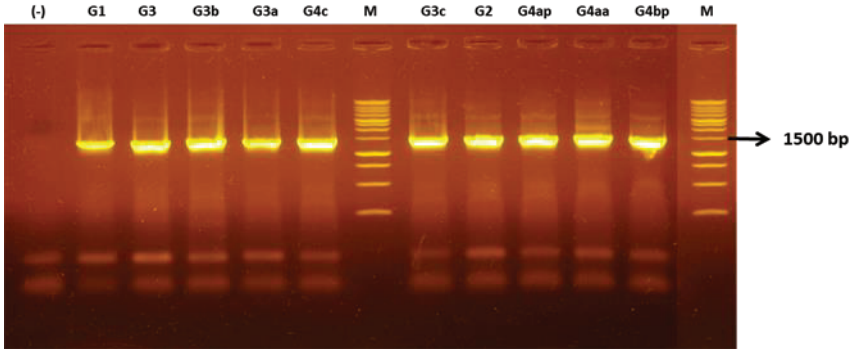


FIGURE 5. PCR Electrophoresis of Isolate Pure Colonies

Remarks; M: 1000 bp markers, (-): Negative Control (without DNA template)

G1, G3, G3b, G3a, G4c, G3c, G2, G4a_p, G4a_a, and G4b_p: Isolate DNA Samples

DNA isolation was carried out using the boiling lysis method. Amplification of DNA of isolates by PCR and visualized it by electrophoresis. Electrophoresis results of isolate DNA samples are presented in Figure 5. From the electrophoresis results of Isolates DNA that have been amplified using PCR, a band with an approximate size of 1500 bp is obtained. The band size prediction shown in the electrophoresis results is the same as the size of 16S rRNA bacteria in the rhizosphere area such as *Azotobacter*, *Azospirillum*, and *Rhizobium* which is about ± 1500 bp. The PCR results were sent to Macrogen for sequencing and then analyzed by comparing it with both NCBI and EzBioCloud Database. The best hits (matched) bacteria strain with its scoring parameter is presented in Table 4 and Table 5 below.

Based on the results of BlastN conducted from sequencing data, the database observed that the isolate sequence was not complete (partial sequence). In Table 4 and Table 5, it is also observed that there are differences in base length between isolates and best hits this affects the accuracy of the prediction of strain isolates. This study used a primary set of 785F 5 '(TTA GAT ACC CTG GTA ARF) 3' and 907R 5 '(CCG TCA ATT CMT TTR AGT TT) 3'. For more accurate results, usually 3 to 4 primary sets can be used, and its results must be compiled together to complete the area that is not complete in the single PCR results. In this study, it is not yet known that specific biochemical tests capable of distinguishing the best hits strains of biochemical characteristics, so that the closest possible use of strains is based on sequencing, Gram staining and *in vitro* test data. G1 isolates have characteristics similar to G2, G3a and G3 isolates. Based on the results of BlastN on NCBI, the top hits are *Beijerinckia fluminensis* strains of UQM 1685 with similarities 96%, 98%, 98%, and 99%, respectively. Whereas based on the EzBioCloud database, the top hits are *Rhizobium radiobacter* ATCC 19358 with similarities of 95.44%, 99.26%, 98%, and 99%, respectively. Based on the results of the reclassification by Oggerin et al. [14] on *Beijerinckia fluminensis* strains of UQM 1685 and *Rhizobium radiobacter* ATCC 19358, it was found that *Beijerinckia fluminensis* strain UQM 1685 was considered as *Rhizobium radiobacter* ATCC 19358 because it has the same characterization based on chemotaxonomy and phenotypic. Based on the data obtained in this study, it was suspected that isolates G1, G2, G3a and G3 were *Beijerinckia fluminensis* strains of UQM 1685, this assumption was based on similarities in possessed characteristics, including having stem cell forms, Gram-negative, not forming spores, isolated from the area rhizosphere of rice plants, and has the ability to fix nitrogen [15].

TABLE 4. The 16S rRNA Comparison Results with BlastN NCBI and EzBioCloud Database

Isolates	BlastN NCBI Results				
	Best Hits Strain	Length (bp)	Accession Number	Query Cover	Identity
G1 (1522 bp)	*Beijerinckia fluminensis* UQM 1685	1448	NR_116306.1	91%	96%
G2 (1366 bp)	*Beijerinckia fluminensis* UQM 1685	1448	NR_116306.1	99%	98%
G3 (1360 bp)	*Beijerinckia fluminensis* UQM 1685	1448	NR_116306.1	99%	99%
G3a (1354bp)	*Beijerinckia fluminensis* UQM 1685	1448	NR_116306.1	100%	98%
G3b (1420bp)	*Achromobacter insuavis* LMG 26845	1391	NR_117706.1	100%	98%
G3c (1422 bp)	*Rhizobium pusense* NRCPB10	1379	NR_116871.1	99%	96%
G4a$_a$ (1433 bp)	*Achromobacter insolitus* LMG 6003	1473	NR_025685.1	99%	97%
G4a$_p$ (1372bp)	*Rhizobium pusense* NRCPB10	1379	NR_116871.1	99%	98%
G4b$_p$ (1388 bp)	*Rhizobium pusense* NRCPB10	1379	NR_116871.1	100%	97%
G4c (1371 bp)	*Rhizobium pusense* NRCPB10	1379	NR_116871.1	100%	99%

TABLE 5. The 16S rRNA Comparison Results with EzBioCloud Database

Isolates	EzBioCloud Results				
	Best Hits Strain	Length (bp)	Accession Number	Completeness	Similarity
G1 (1522 bp)	*Rhizobium radiobacter* ATCC 19358	1363	AJ389904	99.8%	95.44%
G2 (1366 bp)	*Rhizobium radiobacter* ATCC 19358	1363	AJ389904	95.9%	99.26%
G3 (1360 bp)	*Rhizobium radiobacter* ATCC 19358	1363	AJ389904	96.0%	99.03%
G3a (1354bp)	*Rhizobium radiobacter* ATCC 19358	1363	AJ389904	94.8%	98.07%
G3b (1420bp)	*Achromobacter insuavis* LMG 26845	1391	HF586506	95.6%	99.35%
G3c (1422 bp)	*Rhizobium pusense* LMG 25623	1363	jgi. 1102370	97.7%	97.45%
G4a$_a$ (1433 bp)	*Achromobacter insolitus* DSM 23807	1391	CP019325	95.8%	98.63%
G4a$_p$ (1372bp)	*Rhizobium pusense* LMG 25623	1363	jgi. 1102370	96.8%	99.12%
G4b$_p$ (1388 bp)	*Rhizobium pusense* LMG 25623	1363	jgi. 1102370	96.7%	97.42%
G4c (1371 bp)	*Rhizobium pusense* LMG 25623	1363	jgi. 1102370	97.2%	99.49%

Based on sequencing data, the top hits of G3b isolates in the NCBI and EzBioCloud databases are *Achromobacter insuavis* LMG 26845, but the cell form of G3b isolates is not the same as the *Achromobacter insuavis* LMG 26845 cell form that is basil with absolute aerobic properties. While G3b isolates are able to live in semi-aerobic conditions on semi-solid NFb media with coma cell forms. G3b isolate is thought to be *Azospirillum* sp. with Gram-negative characteristics, coma cell form, isolated from the rhizosphere area, not producing spores, living in semi-aerobic conditions, and possessing the nitrogen-fixing ability [16]. Based on sequencing data, the top hits of $G4a_a$ isolates in NCBI and EzBioCloud databases are *Achromobacter insuavis* with similarities of 97% and 98.6%, respectively. This estimation is reinforced by the data of bacterial morphology and Gram staining test presented in Table 4 and Table 5, which are in accordance with the characteristics of the *Achromobacter insuavis* bacteria, which are Gram-negative, stem, motile, and many are found in the soil [17].

The isolates of G4c, G3c, $G4a_p$, and $G4b_p$ are thought to be *Rhizobium* with similarities of 99%, 96%, 98%, and 97%, respectively in NCBI databases, and percent similarities to EzBioCloud databases 97.45%, 99%, 97.4%, and 99.5%, respectively. This allegation is reinforced by the data of bacterial morphology and Gram staining test presented in Table 2, which are in accordance with the characteristics of *Rhizobium presence* bacteria which are Gram-negative, rod-shaped, and have flagellum [18]. Specific biochemical tests are needed to distinguish between types of bacteria, but currently, there is no known biochemical test that is suitable to do. Based on the overall data obtained in this study, it is known that G4c isolates have the greatest potential as a plant growth booster agent. G4c isolate has the ability to dissolve phosphate, produce siderophore, and is able to produce IAA that is consistent with high concentrations compared to other isolates that were able to be isolated in this study. From the results of sequencing, it is thought that G4c isolates are Rhizobium bacteria due to the similarity of Rhizobium's ability to produce phytohormones and act as phosphate solvent agents.

In the research conducted by Sukweenadhi [3], *Sphingomonas panaciterrae* and *Paenibacillus yonginensis* which obtained from ginseng field land in South Korea have the potential as a plant growth promoting activity with the ability of isolates to produce IAA, siderophores, and phosphate dissolution. In the research conducted by Ullah et al. [4], PGPR isolates obtained from *Zea mays* were able to produce IAA, gibberellins and were able to accumulate heavy metals in the soil. From the results of isolation and purification in the rhizosphere area of red rice plants CV. Gecko isolates were obtained by G4c isolates which are thought to be *Rhizobium pusense* bacteria, potentially as plant growth booster bacteria with the ability to produce IAA, siderophores, and phosphate dissolution. The superior isolates obtained may have the potential as single inoculants for plant growth booster agents or combined with other bacteria that are synergistic which can be used as one of the inoculants in making organic fertilizers to produce a better effect on plant growth, for further research is needed.

CONCLUSION

There were 10 bacterial isolates that were successfully purified from the rhizosphere area of the Barak Cenana red rice. G4c bacterial isolates have the greatest potential as bacterial-growth-growth plants, based on the ability of the isolates to produce IAA, siderophores, and phosphate solubilization. For further research, media other than NFb should be used in isolating the bacteria from the rhizosphere area of the Barak Cenana red rice, to obtain a type of bacteria other than the bacteria that was successfully obtained in this study. It would be interesting to test the ability of bacteria to produce nitrogen as well. In addition, the inhibition of growth of pathogens should be carried out to support the isolate data as a PGPR agent. For more specific identification of bacteria from the results of this study, it is recommended to carry out biochemical tests that are specific to the strains closest to the results of BlastN, so that more accurate results will be obtained. In addition, the addition of the use of a primary set of 16S rRNA sequencing can also be done to get a complete sequence for molecular identification. Based on *in vitro* testing data carried out, in further research it is better to do direct testing on plants, either model plants first or field tests on commodity crops.

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MAKALAH PENELITIAN

SURAT TUGAS

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